Gene Therapy for Lung Disease: Hype or Hope?

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Gene therapy, the treatment of any disorder or pathophysiologic state on the basis of the transfer of genetic information, was a high-priority goal in the 1990s. The lung is a major target of gene therapy for genetic disorders, such as cystic fibrosis and α_1-antitrypsin deficiency, and for other diseases, including lung cancer, malignant mesothelioma, pulmonary inflammation, surfactant deficiency, and pulmonary hypertension. This paper examines general concepts in gene therapy, summarizes the results of published clinical trials, and highlights areas of research aimed at overcoming challenges in the field. Although progress has been slower than anticipated, gene transfer has been safely achieved in patients with lung diseases. Recent advancements in understanding of the molecular basis of lung disease and the development of improved vector systems make it likely that gene therapy will be an important tool for the 21st-century clinician.

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For definitions of terms, see Glossary at end of text.

Approximately 10 years ago, advances in molecular genetics and gene transfer technology made possible the development of gene therapy, modification of the genetic makeup of cells for therapeutic purposes. Although gene therapy was originally proposed for treatment of inherited recessive disorders in which transfer of a normal copy of a single defective gene might prevent the development of disease or slow its progression (1), it soon became clear that reasonable targets for gene therapy extended beyond traditional genetic disorders into the realm of acquired diseases, such as vascular diseases and cancer. The concept of gene therapy now encompasses the treatment of any pathophysiologic state on the basis of the transfer of genetic material, including complementary DNA, full-length genes, RNA, or oligonucleotides.

Lung diseases are prominent among the disorders for which human gene therapy protocols have been approved (2). From a technical perspective, delivery to the lung can be accomplished by multiple approaches—through the airway, by direct transthoracic injection, or through the dual pulmonary blood supply. From a disease perspective, the genetic basis for the two most common fatal inherited lung disorders, cystic fibrosis and α_1-antitrypsin deficiency, is known, and current treatment regimens are supportive but not curative (3, 4). Several other pulmonary disorders, such as lung cancer, mesothelioma, pulmonary vascular diseases, radiation-induced lung injury, transplantation-induced lung injury, asthma, and pulmonary inflammation, are potential targets for gene therapy.

Study Selection

English-language articles were identified by searching the MEDLINE database from 1966 to the present using the terms *gene therapy, lung, lung cancer, cystic fibrosis, α_1-antitrypsin deficiency, mesothelioma, retrovirus, adenovirus, adeno-associated virus, lentivirus, vaccinia virus,* and *liposome*. Selected references cited in identified articles were reviewed. We focused on articles that described clinical trials. Because of space limitations, the references include excellent review articles that discuss the many in vitro and animal model experiments that form the scientific background for the clinical studies.

Vectors Used in Gene Therapy

Efficient gene transfer is a basic requirement for effective gene therapy. Various viral and nonviral gene transfer vectors are currently available (5–8). As summarized in **Table 1**, each vector has certain advantages in DNA-carrying capacity, types of cells targeted, efficiency of in vivo gene transfer, duration of expression, and induction of inflammation. It has become clear that no one gene delivery strategy will be suitable for all candidate disorders.

The most widely used vector in lung gene therapy has been replication-incompetent recombinant adenovirus. This vector system offers many advantages, including high-efficiency transduction in a wide variety of target cells (including nondividing cells) and high levels of expression of the delivered transgene (8, 9). Of note, these vectors are stable in vivo, permitting direct delivery of the gene to many tissue sites, including the lung parenchyma and pleural space. The two primary disadvantages of adenoviruses are that they result in only transient gene expression and that when virions are used for direct in vivo applications, they elicit a prominent local

Table 1. Characteristics of Gene Therapy Vectors

Vector	DNA-Carrying Capacity, *kilobase*	Cell Rage	Efficiency of In Vivo Gene Delivery	Duration of Expression	Co-Transfer of Viral Gene Elements?	Inflammatory Response
Retrovirus	<8	Replicating cells only	Low	Stable	Yes	Low
Adenovirus	7–8	Most cells	Moderate	Transient	Yes	High
Adeno-associated virus	<5	Most cells	Low	Stable	Yes	Low
Lentivirus	<8	Many nondividing cells	Low	Stable	Yes	Low
Liposome	>10	Most cells	Low	Transient	No	Low to moderate
Molecular conjugate	>10	Most cells	Low	Transient	No	Low to moderate

and systemic inflammatory response. This inflammatory response, which includes an early "innate" immune response resulting in cytokine release and a late "acquired" immune response resulting in the generation of neutralizing antiadenoviral antibodies and cytotoxic T lymphocytes (10–15), has been the primary source of adenoviral vector toxicity and has limited the amount of vector that can be delivered.

Gene Therapy for Inherited Pulmonary Disorders

Cystic Fibrosis

Cystic fibrosis results from mutations in the gene that encodes the cystic fibrosis transmembrane conductance regulator (CFTR) (4). The CFTR seems to play an important role in regulating the vectorial movement of fluid and electrolytes across various tissues and probably contributes to processes as diverse as mucus secretion, sialylation of surface receptors, sulfation of glycoconjugates, and local host defense (4, 16, 17). An early finding that made gene therapy for cystic fibrosis a potentially achievable goal was the observation that in a polarized epithelial sheet, expression of CFTR in as few as 6% to 10% of airway epithelial cells lacking CFTR restored normal chloride transport properties (18, 19). Because respiratory problems are responsible for most of the morbidity and mortality in persons with cystic fibrosis, the bulk of gene therapy research in cystic fibrosis has focused on methods of transfer to airway epithelium. Three vector systems have been used in clinical trials to date: adenovirus, adeno-associated virus, and liposomal/DNA complexes.

Adenovirus

To date, results of seven phase I clinical trials that used an adenoviral vector carrying the *CFTR* transgene have been published (**Table 2**). In the initial studies, the *CFTR* transgene was delivered by direct instillation in the nose or lung (20–22). In a randomized, vehicle-controlled, blinded study in 12 patients, Knowles and coworkers (23) studied gene transfer to nasal epithelium. Although no significant side effects occurred, gene transfer was inefficient and significant inflammation was noted at a dose of 2×10^{10} plaque-forming units, an estimated multiplicity of infection of 1000 (that is, 1000 infectious units of virus per cell). Furthermore, the investigators could not detect correction of sodium or chloride transport as measured by using nasal electrical potential difference. Zabner and colleagues (24) reported on the effect of repeated administration of recombinant adenoviral vector CFTR applied to nasal epithelium. Six patients received five escalating doses of adenovirus over at least 28 days. A humoral immune response was stimulated, gene transfer was inefficient, and functional correction was variable and seemed to be attenuated by subsequent administration of vector. To improve gene transfer, Bellon and coworkers (25) used an adenoviral vector CFTR (identical to that used by Crystal and associates [21]) to deliver aerosolized recombinant CFTR sequentially to the nasal epithelium and lung in 6 patients. Recombinant transcripts and CFTR protein were detected up to 15 days later in epithelial cells harvested from the nose and lung, although the amounts detected were low. In an effort to reduce immunogenicity, Zuckerman and colleagues (26) delivered a more crippled form of recombinant adenovirus, in which the E1 and E4 regions were deleted, to the lower respiratory tracts of 11 patients. As in the previous studies, gene transfer efficiency was low; gene transfer was detected in less than 1% of harvested bronchial epithelial cells at 4 days and 0% at 42 days. Systemic humoral immune responses to adenovirus were relatively mild, but cell-mediated responses (measured by lymphoproliferative responses to adenovirus) were stimulated in all patients. Dose-limiting toxicity was noted at 10^{11} viral particles.

Adeno-Associated Virus

Adeno-associated viruses have been considered as gene therapy for cystic fibrosis for some time, but they were only recently used in human trials because of technical constraints related to production issues (32, 33). Adeno-associated virus is attractive because preclinical studies have suggested that long-term (up to 6 months) transgene expression can be obtained (32, 34) with little inflammatory response

(35). Two phase I clinical studies have been approved by the National Institutes of Health, and to date, a summary of the results of one has been published. The first study (27, 28) involved a randomized, nonblinded, dose-escalation protocol in which a vector suspension was instilled into the maxillary sinuses of 10 patients with cystic fibrosis who had previously undergone bilateral endoscopic antrostomies (**Table 2**). Vector-derived DNA was detected in all participants who received more than 10^4 replication units. Although vector-derived RNA could not be detected in any of the specimens, transepithelial potential difference responses consistent with CFTR-mediated chloride transport were observed up to 14 days after instillation in patients receiving more than 5×10^4 replication units. The second study, which is ongoing, is examining dose-escalated administration of adeno-associated virus CFTR, both intranasally and to a single lobe of the lung (by bronchoscope); toxicity is the primary end point (36). Although the results from the sinus study are encouraging, questions remain about the efficiency of transduction of differentiated respiratory epithelium (37–39) and the long-term safety of adeno-associated virus vectors (40).

Liposomes

The liposomal vectors in clinical use are generally composed of cationic lipids mixed with cholesterol and dioleoylphosphatidylethanolamine. The liposome/DNA complexes were originally thought to be less immunogenic than viral vectors, although as more efficient complexes have been designed, it seems that these vectors can also generate significant inflammatory responses, probably because of the inherent immunogenicity of bacterial-derived DNA (41, 42). The results of three clinical trials (**Table 2**) have been published to date. Caplen and colleagues (29) conducted a double-blind, placebo-controlled study in which 9 patients with cystic fibrosis received nasal spray application of liposome/DNA complex and 6 such patients received control liposomes. The investigators observed vector-derived RNA in 5 of 8 patients and a transient, partial normalization of the response to low chloride perfusion during nasal potential difference measurements in the treated patients with cystic fibrosis (compared with controls without the disease). Another study of nasal application in 12 participants with cystic fibrosis also used a double-blind, placebo-control design (30). Functional expression of *CFTR* was transiently detected by using nasal potential difference in 2 of the 8 treated patients, and some evidence of functional gene transfer on a halide-sensitive fluorochrome dye technique was seen in 5 of the treated patients (43). A third double-blind, placebo-controlled trial studied a total of 16 patients using plasmid DNA at a single dose of 0.4 mg (31). No significant functional correction was detected; however, vector DNA was detected in 7 of 8 patients at 28 days, and vector-derived RNA was detected in 2 patients (up to 7 days). Although none of these trials has reported immunologic data, this localized instillation of liposome/DNA complexes seemed to be well tolerated. Several trials of liposome vectors are ongoing in the United States (44, 45).

α_1-Antitrypsin Deficiency

α_1-Antitrypsin deficiency is an autosomal recessive disease characterized by the development of panacinar emphysema and cirrhosis of the liver (46). Pulmonary disease results from deficient serum and lung levels of α_1-antitrypsin antiprotease. Thus, after the α_1-antitrypsin gene was cloned, α_1-antitrypsin deficiency became a logical candidate for gene transfer (47, 48).

Many approaches have been examined in animal model systems, including injection of cells that were modified ex vivo into the peritoneal cavity of animals (49), direct in situ modification of hepatocytes (the cell usually responsible for α_1-antitrypsin biosynthesis) by using an adenoviral vector (50), in situ transduction of hepatocytes by using vascular delivery of retroviral vectors (51), and direct augmentation of α_1-antitrypsin levels in epithelial lining fluid by using recombinant adenoviruses (52) or liposomal vectors (53). Unfortunately, in all of these systems, α_1-antitrypsin levels were only transiently increased and were below what would be required for physiologic correction. Two potentially promising developments in this area are a new α_1-antitrypsin–encoding adenoviral vector in which all viral coding sequences have been deleted (54) and an adeno-associated virus vector encoding α_1-antitrypsin that can be injected intrahepatically (55, 56). No clinical trials of gene therapy for α_1-antitrypsin deficiency have been published to date; however, studies using these new technologies will probably be forthcoming.

Gene Therapy for Pulmonary Vascular and Other Lung Disorders

Injury to the pulmonary endothelium plays an important role in the pathogenesis of a wide variety of diseases and syndromes, including pulmonary edema, the acute respiratory distress syndrome, sepsis, lung transplant rejection, radiation-induced lung injury, pneumonia, dissemination of malignant cells in the lung, oxidative lung injury, lung ischemia-reperfusion injury, pulmonary hypertension, lung inflammation, and pulmonary fibrosis (57). The pul-

Table 2. Clinical Trials of Gene Therapy for Cystic Fibrosis*

Study, Year (Reference)	Vector	Site of Administration	Patients, *n*	Highest Dose	Gene Transfer
Zabner et al., 1993 (20)	Adenovirus (serotype 2); recombinant adenovirus missing the E1 region of the virus	Nose	3	5×10^7 IU	DNA detected (data not shown); nasal potential difference (3 of 3 patients showed some normalization of the baseline potential difference and response to agonist)
Crystal et al., 1994 (21)	Adenovirus (serotype 5); recombinant adenoviruses missing the E1 and E3 region of the virus	Lung, nose	4	Lung, 2×10^9 PFU; nose, 2×10^7 PFU	DNA detected (up to 15 days after administration); messenger RNA (up to 9 days); protein (up to 4 days)
Hay et al., 1995 (22)	Adenovirus (serotype 5); recombinant adenoviruses missing the E1 and E3 region of the virus	Nose	9	$2 \times 10^{8.5}$ PFU	Nasal potential difference
Knowles et al., 1995 (23)	Adenovirus (serotype 5); recombinant adenoviruses missing the E1 and E3 region of the virus	Nose	12	2×10^{10} PFU	DNA detected (up to 8 days after administration); messenger RNA (up to 6 days); nasal potential difference (no significant changes)
Zabner et al., 1996 (24)	Adenovirus (serotype 2); recombinant adenoviruses missing the E1 or E4 region of the virus	Nose	6	1×10^{10} IU	Nasal potential difference (small change in potential difference during low chloride/terbutaline perfusion up to 7 days after administration)
Bellon et al., 1997 (25)	Adenovirus (serotype 5); recombinant adenoviruses missing the E1 and E3 region of the virus	Nose, lung	6	5.4×10^8 PFU	RNA detected in 6 of 6 patients (nasal) and 1 of 6 patients (bronchial) up to 15 days after administration; protein detected in 6 of 6 patients (nasal) and 2 of 6 patients (bronchial) up to 15 days
Zuckerman et al., 1999 (26)	Adenovirus (serotype 5); recombinant adenoviruses missing the E1 and E4 region of the virus	Lung	11	2.1×10^{11} particles (about 2.1×10^9 PFU)	RNA detected in 6 of 11 patients 3 days after administration
Wagner et al., 1998 (27, 28)	Adeno-associated virus	Maxillary sinus	10	10^5 replicating units	DNA but not RNA detected at doses $>10^4$ replicating units; some transepithelial potential difference responses observed at highest doses
Caplen et al., 1995 (29)	Liposome/DNA conjugate	Nose	9	0.3 mg plasmid DNA	RNA detected in 5 of 8 patients; 20% normalization of potential difference measurements
Gill et al., 1997 (30)	Liposome/DNA conjugate	Nose	12	0.4 mg plasmid DNA	Molecular analysis not done; some functional correction in 5 of 8 patients
Porteous et al., 1997 (31)	Liposome/DNA conjugate	Nose	16	0.4 mg plasmid DNA	DNA detected in 7 of 8 patients 28 days after administration; RNA detected in 2 patients

* PFU = plaque-forming units.

monary endothelium is thus an attractive target for gene therapy.

To date, success has been limited in obtaining stable pulmonary vascular gene transfer in vivo. Liposomal vectors seem to transduce lung tissue (presumably lung endothelium) preferentially after intravenous injection; however, expression is low compared with viral vectors (58). Systemic activation of cytokines as part of the inflammatory response to injected bacterial DNA used in the more potent liposomal complex is proving to be problematic (59). Viral approaches have also met with limited success thus far. Investigators have not been able to use adenoviral vectors to efficiently transfer marker genes to the pulmonary circulation without stopping blood flow, as is done with catheters directed to the systemic circulation. Gene expression has been inconsistent and transient and has been accompanied by induction of strong inflammatory reactions (58).

Despite these limitations, some attempts at vascular gene therapy have been pursued in animal models. Conary and colleagues (60) delivered the prostaglandin G/H synthetase gene into the pulmonary artery of rabbits by using cationic liposomes. Expression of this enzyme might be useful in regulating pulmonary hypertension. Other approaches, such as delivery of nitric oxide synthetase, are also under investigation for the treatment of pulmonary hypertension (61). Ex vivo liposomal vascular gene transfer has also shown some promise in lung transplantation in rats (62).

An area of much interest is delivery of antioxidants or anti-inflammatory genes that could ameliorate ischemia-reperfusion injury, transplant rejection, or other inflammatory lung diseases (63). Accordingly, efforts have been made to deliver antioxidant enzymes, such as catalase or superoxide dismutase, to lung cells. Erzurum and colleagues (64) were among the first to show that adenoviral vectors

Table 2—*Continued*

Toxicity	Immunology	Comments
No vector-related adverse events noted	Not done	
One patient who received the highest dose developed infiltrate, fever, and hypotension	In 3 of 4 patients, levels of antiadenovirus antibodies but not neutralizing antibodies increased; serum levels of interleukin-6 transiently increased	The patient with toxicity received vector in 20-mL volume; volume was subsequently reduced
No vector-specific events noted	Not done	
At the highest dose, 2 of 3 patients had nasal mucosal inflammation	One patient who received the highest dose had a 16-fold increase in serum level of neutralizing antibodies	
No adverse events noted	Three of 6 patients had a fourfold increase in levels of neutralizing antibodies, and all showed an increase in levels of adenovirus-specific antibodies	Gene transfer seemed to become less efficient with subsequent administration
No vector-specific events noted	No increase in levels of adenovirus-specific antibodies or neutralizing antibodies, lymphoproliferative response, or inflammatory cytokines in blood or bronchoalveolar lavage samples	
Two of 3 patients who received the highest dose developed a focal infiltrate in the area of dosing	Modest increases in serum neutralizing antibodies; lymphoproliferative responses were significant at all doses	
No vector-specific events noted	Not reported	
No vector-specific events noted	Not reported	
Transient earache in 1 patient	Not reported	
No vector-specific events noted	Not reported	

could deliver the catalase gene to pulmonary vascular endothelium. Danel and associates (65) extended this approach by intratracheal delivery of an adenovirus-encoding superoxide dismutase in a mouse model of hyperoxia-induced oxidative lung injury and reducing lung injury. Similarly, Epperly and colleagues (66) intratracheally delivered human manganese superoxide dismutase by plasmid or adenovirus to the lungs of mice subjected to γ-irradiation and observed a decrease in the late effects of exposure (alveolitis and fibrosis).

Aerosolization of vectors may improve delivery to airway epithelium (67), and this seems to be an ideal approach for surfactant replacement. On the basis of this concept, two groups have demonstrated that adenoviral gene transfer may be used to express intrapulmonary surfactant apoproteins (68, 69).

Finally, gene therapy approaches for asthma have been proposed. As shown in a recent in vitro study, the glucocorticoid receptor gene seems to be a candidate for gene therapy in asthma (70). Animal studies using delivery of Th1-type cytokines, such as interleukin-12 or interferon-γ, have led to decreased airway reactivity in allergen-induced airway hyperresponsiveness (71, 72). Given the existence of effective and safe treatments for asthma, however, these preclinical experiments may prove more useful for increasing our understanding of pathogenetic mechanisms rather than for potential clinical application.

Gene Therapy for Thoracic Cancers

Recent advances in the understanding of growth factors, molecular oncology, and tumor immunology have provided the rationale for several strategies for cancer gene therapy (73). Some of these approaches are being tested in clinical trials in patients with

Table 3. Phase I Clinical Trials of Gene Therapy for Thoracic Tumors*

Study, Year (Reference)	Vector	Target	Route of Administration	Patients, *n*	Highest Dose
Roth et al., 1996 (74)	Retrovirus encoding p53 gene	Non–small-cell lung carcinoma pulmonary nodules	Endobronchially or by CT-guided needle	9	5×10^8 CFU
Tursz et al., 1996 (75); Gahéry-Ségard et al., 1997 (76)	Adenovirus (serotype 5) encoding β-galactosidase gene	Non–small-cell lung carcinoma pulmonary nodules	Endobronchially	10	10^9 PFU
Schuler et al., 1998 (77)	Adenovirus (serotype 5) encoding p53 gene	Non–small-cell lung carcinoma pulmonary nodules	Endobronchially or by CT-guided needle	15	10^{10} PFU (7.5×10^{12} particles)
Swisher et al., 1999 (78)	Adenovirus (serotype 5) encoding p53 gene (patients were also given cisplatin)	Non–small-cell lung carcinoma pulmonary nodules	Endobronchially or by CT-guided needle	28	10^{11} PFU given monthly up to 6 times
Sterman et al., 1998 (79); Molnar-Kimber et al., 1998 (15)	Adenovirus (serotype 5) encoding HSV*tk* gene	Malignant pleural mesothelioma	Intrapleural injection	21	1×10^{12} PFU
Mukherjee et al., 1997 (80); Robinson et al., 1998 (81)	Replication-deficient vaccinia virus expressing interleukin-2	Chest wall masses in patients with malignant mesothelioma	Intratumoral injection	6	10^7 PFU
Schwarzenberger et al., 1998 (82)	Retrovirally transduced irradiated tumor cells expressing HSV*tk*	Malignant pleural mesothelioma	Intrapleural injection	14	5×10^9 cells

* CFU = colony-forming units; CT = computed tomography; HSV*tk* = herpes simplex thymidine kinase; PFU = plaque-forming units; RT-PCR = reverse transcription polymerase chain reaction.

lung cancer and malignant mesothelioma. A summary of published clinical trials for thoracic cancers is shown in **Table 3**. Because none of the currently available vectors distribute systemically, therapeutic approaches have focused on treatment of localized disease or induction of an immune response capable of eliminating distant tumor cells.

Tumor Suppressor Gene Replacement Therapy (p53)

One of the most common genetic abnormalities in non–small-cell lung cancer is mutation of the tumor suppressor gene p53 (73). Preclinical work showed that delivery of wild-type p53 to lung cancer cell lines with deleted or mutated p53 caused some degree of apoptosis (especially in combination with the antitumor drug cisplatin) (83). In animal models, transduction of a subset of cells in tumors with vectors encoding wild-type p53-induced tumor regression (84), suggesting the existence of a "bystander effect" by which transduced cells inhibit the growth of nontransfected cells. Although the mechanism of this effect is still not completely defined (85), possible pathways include release of angiogenesis inhibitors (86), activation of the Fas/Fas ligand system (87), and immunologic response.

Three phase I clinical trials in humans using gene transfer of p53 have been reported (**Table 3**). In all three trials, viral vectors encoding wild-type p53 were injected into the tumors of patients with non–small-cell lung cancer by means of a bronchoscope or percutaneous computed tomography–guided needles. In the first trial (74), a retroviral vector was used. The treatments were well tolerated, with minimal side effects. Some evidence of gene transfer was noted in patients given higher doses, and a subgroup of patients showed evidence of stabilization or regression of the injected tumors; however, no effects on noninjected tumors were noted. In the other two trials, adenoviral vectors were used (77, 78). Swisher and colleagues (78) used monthly injections of an adenovirus p53 vector in conjunction with administration of cisplatin. Treatment was well tolerated, and despite repeated doses of vector that induced antiadenoviral antibodies, gene transfer was detected in most patients receiving higher doses. Transient local control was observed in one third to one half of the participants.

Intratumoral injection of adenoviral p53 for the treatment of lung cancer thus seems to be well tolerated, safe, and perhaps capable of local antitumor effects. However, because of the lack of systemic efficacy, the ultimate clinical utility of this approach will probably be limited to the few patients with nonresectable disease that is not or cannot be controlled with local radiation therapy.

Suicide Gene Therapy

Another approach to the treatment of localized cancer is suicide gene therapy. In this therapy, a gene encoding an enzyme that catalyzes conversion of a normally nontoxic agent to a toxic substance is delivered to tumor cells. The toxic substance then eradicates tumor cells (88). The most widely used strategy has been introduction of the thymidine kinase gene from herpes simplex virus (HSV*tk*) into

Table 3—*Continued*

Gene Transfer	Toxicity	Immunology	Clinical Response
Not examined; increased apoptosis noted in 6 patients	None	Not done	Three patients showed evidence of nodule regression, although high volumes of fluid were injected
β-Galactosidase staining seen in 7 patients	Minor: moderate bleeding and fever	Strong antiadenovirus antibodies and anti–β-galactosidase antibodies generated	Temporary local nodule regression in 6 patients
Vector sequences detected by RT-PCR at higher doses	None	Antiadenovirus antibodies increased after vector administration	Transient local control seen in 4 patients; no response in nontreated sites
Vector sequences detected in most patients; some increase in p53 immunostaining or apoptosis also noted	Minor: fever, chills	Very high titers of antiadenovirus antibodies induced, including neutralizing antibodies	Reported partial responses in 8 patients; disease stabilization in 64%; and disease progression in 28%
Successful transgene protein expression in 11 patients, including detection by immunohistochemistry	Minor: fever, chills, skin blisters	Strong humoral and cellular antiadenovirus immune responses generated, including neutralizing antibodies	No uniform responses, although partial tumor regressions noted in patients receiving high doses
Vector DNA detected transiently in all patients by using RT-PCR	Minor: fever	Significant increase in serum antivaccinia antibody titer	None
Not applicable	Minor	Increases in the percentage of $CD8^+$ T cells in pleural fluid	None

mammalian cells. This enzyme converts the normally nontoxic nucleoside analogue ganciclovir to a toxic form. The success of the HSV*tk*–ganciclovir approach is bolstered significantly by the presence of a "bystander effect" (89). This involves the transfer of toxic metabolites from transduced cells to nontransduced cells through gap junctions (90) and the generation of an immunostimulatory environment in vivo that enhances immune responses (91).

On the basis of success in animal models, Sterman and colleagues (79) conducted a phase I clinical trial of a replication-incompetent adenoviral vector encoding HSV*tk* that was delivered intrapleurally to 21 patients with pleural mesothelioma. After vector instillation, patients received systemic ganciclovir therapy for 2 weeks. As shown in **Table 3**, dose-limiting toxicity was not reached; side effects were minimal; and dose-related gene transfer was confirmed in 11 of 20 evaluable patients, in whom gene transfer was clearly detectable on immunostaining at tumor surfaces that penetrated up to 30 to 50 cell layers (79). However, strong antiadenoviral immune responses, including high titers of neutralizing antibody and proliferative T-cell responses, were generated with no obvious adverse clinical effects (15). Although clinical responses were not consistently seen, 1 patient remains tumor-free 3 years after treatment and partial tumor regression was observed in several of the patients who received the higher doses of vector. Further modifications to the study protocols include escalation of the dose of ganciclovir, multiple administrations of vector, and combination of vector instillation with surgical tumor debulking.

Immunogenetic Therapy

One attractive approach to the treatment of disseminated cancer is to make a subset of tumor cells more recognizable to the immune system, thus allowing widespread immune-mediated tumor destruction. Various gene therapy approaches have been developed with this goal in mind (92).

On the basis of the idea that expression of a foreign transgene might augment antitumor immunity, a phase I trial studied the transfer of the bacterial gene β-galactosidase into lung cancer tumor nodules by using replication-incompetent adenovirus (75, 76) (**Table 3**). Ten patients were injected with increasing doses of the vector by using a bronchoscope. Evidence of transgene expression in the nodules was obtained, and strong antiadenoviral and antitransgene immune responses (both humoral and cell mediated) were noted. Somewhat surprisingly, some localized antitumor responses were observed, suggesting an antitumor immunologic response.

Ex vivo approaches are also being developed. For example, on the basis of animal data (93) and encouraging phase I data in prostate cancer (94), a multicenter immunotherapy trial in lung cancer has been established in which tumor cells will be harvested, infected ex vivo with adenovirus-encoding granulocyte-monocyte colony-stimulating factor, and reinjected intradermally into patients.

Results of a phase I clinical trial in pleural mesothelioma that used a recombinant vaccinia virus expressing the human interleukin-2 gene have been reported (80, 81) (**Table 3**). The vaccinia virus–interleukin-2 vector was injected repeatedly into

palpable chest wall masses of six patients with advanced-stage malignant mesothelioma. Toxicity was minimal, and no clinical or serologic evidence of spread of vaccinia virus to patient contacts was seen. No patient had significant tumor regression, and minimal intratumoral cellular immune responses were detected. In future gene therapy approaches to mesothelioma, vaccinia virus–interleukin-2 may show improved efficacy in a more replication-competent form or as part of a "cocktail" of cytokine genes delivered by way of vaccinia virus (such as interleukin-2, interleukin-12, and granulocyte-monocyte colony-stimulating factor).

Suicide Gene Therapy plus Immunotherapy

Several animal studies have suggested that the combination of adenoviral vectors encoding HSV*tk* with adenoviral vectors expressing certain cytokines (for example, interleukin-2 or interferon-α) can enhance therapeutic efficacy by augmenting antitumor responses (91, 95). No clinical trials using such combinations have yet been reported; however, Schwarzenberger and colleagues (82) reported a phase I clinical trial in patients with malignant mesothelioma in which an irradiated ovarian carcinoma cell line retrovirally transfected with HSV*tk* (PA1-STK cells) was instilled intrapleurally, followed by systemic administration of ganciclovir (**Table 3**). The rationale behind this trial is that the PA1-STK cells will migrate to areas of intrapleural tumor after instillation and will facilitate bystander killing of mesothelioma cells after ganciclovir infusion. To date, 14 patients have been treated. The treatment produced minimal side effects but no obvious clinical responses. Preliminary findings have shown significant increases in the percentage of CD8 T lymphocytes in pleural fluid after instillation of PA1-STK cells (96).

Problems and Future Approaches

Several problems must be overcome before successful gene therapy can become a reality. The major challenge is posed by inefficient gene delivery. With regard to cystic fibrosis, all of the currently available vectors seem to offer relatively poor entry into intact differentiated airway epithelium. Removal of inflammatory mucus, improvement of access of vectors to receptors needed for viral entry, and development of methods to exploit endogenously expressed apical proteins are all possible approaches (97). Even more important will be optimization of existing vectors (such as "gutless" adenoviral vectors that express no endogenous viral genes [54] or improved liposomal preparations) or development of new vectors (for example, lentiviruses, such as feline immunodeficiency virus [98]) that will allow longer-term expression, induce minimal inflammatory responses, and permit easy retreatment. In cancer gene therapy, development of replicating viral vectors that can kill tumors by primary viral lysis or enhanced delivery of therapeutic genes to cancer cells are areas of special interest (99). Promising viruses in this regard are replication-selective adenoviruses (100, 101), the avian Newcastle disease virus (102), or mutants of herpes simplex-1 virus (103).

Another desirable direction in development of improved vectors will be enhanced specificity for target tissues, a feature that will provide important safety factors. One approach to this goal has been to redirect the tropism of adenoviral vectors away from the natural cellular receptor and toward surface proteins that are highly expressed on target cells (104). This strategy could be used to augment gene uptake in lung epithelial cells for use in cystic fibrosis or to enhance tumor cell targeting. Some tumor-cell specificity has been achieved with replicating vectors. Clinical trials currently under way in head and neck cancer, liver metastases, and prostate cancer are using modified adenoviruses that lack the *E1B* gene (a deletion that allows the virus to replicate selectively in tumor cells) or have tumor-selective promoters (for example, prostate-specific antigen) driving key viral genes, such as *E1A* (100, 101).

The interaction of the immune system and gene therapy is under extensive investigation. Suppression or avoidance of immune responses to vectors and transgenes are key goals of therapy for metabolic or genetic diseases, such as cystic fibrosis or α_1-antitrypsin deficiency. Adeno-associated viruses and lentiviruses seem especially promising in this regard. However, manipulation of the recipient's immune system by administration of immunosuppressants (105–107), use of "gutless" adenoviral vectors (54), or coating the adenoviral vector with polyethylene glycol to shield the virus from the immune system (108, 109) are being investigated in animal models and will probably form the basis of future clinical trials.

In contrast, activation of the immune system by using gene therapy is the goal of anticancer immunotherapy. For lung cancer, promising preclinical approaches include transfer of cytokines, such as interleukin-2 (110), interleukin-3 (111), and interleukin-7 (112); intratumoral injection of activated dendritic cells (113); and inhibition of immunosuppressive cytokines, such as prostaglandin E_2, interleukin-10, and transforming growth factor-β (114). Other ideas, such as antiangiogenic approaches (115), antisense strategies, and delivery of genes that allow sensitization to radiation or chemotherapy, are also being studied (92, 116).

Summary

The field of gene therapy is still in its infancy; however, accomplishments thus far have been significant. Genes have been safely and successfully transferred into animals and patients. It is highly likely that in the near future, gene therapy will be shown to have clear efficacy in treatment of such diseases as hemophilia (by using adeno-associated virus vectors) and in stimulation of angiogenesis in peripheral vascular disease and myocardial ischemia. Although only early trials of gene therapy in cancer have been conducted, usually in patients with large tumor burdens and at submaximal doses, hints of efficacy at higher doses of vector in trials for localized malignancy have been seen.

The studies reviewed here demonstrate the first attempts to use gene therapy vectors for diverse inherited and acquired pulmonary diseases. Although none of the diseases studied was "cured," valuable lessons have been learned from these trials, especially in defining the challenges of relatively inefficient and transient delivery of transgene in vivo. Using this knowledge, the second phase of gene therapy research has begun, with a strong focus on improving vector technology. Given the progress thus far, gene therapy will probably become a reality for many lung diseases during the next century.

Glossary

Bystander effect: The effect seen in suicide gene therapy in which not all tumor cells in a solid tumor need to be transduced to achieve efficient killing. For example, herpes simplex thymidine kinase (HSV*tk*)–negative tumor cells in the vicinity of HSV*tk*-positive tumor cells can be killed by exposure to ganciclovir because of transfer of toxic metabolites from transduced cells to nontransduced cells through gap junctions. Bystander effects can also be produced by the generation of an immunostimulatory environment in vivo that enhances immune responses against tumors.

E1A region of adenovirus: The genes in the E1A region of adenovirus are the first to become activated and primarily facilitate viral replication. In the absence of the E1A region of the virus, replication occurs at negligible levels.

E1B region of adenovirus: A set of genes activated very early after adenovirus infection. In the context of adenoviral replication, one of the most important functions of the E1B proteins is to prevent the onset of apoptosis (programmed cell death) in the presence of E1A.

Gene therapy: Transfer of genetic material, including complementary DNA, full-length genes, RNA, or oligonucleotides, into a host with the hope of ameliorating or curing a pathophysiologic state.

"Gutless" adenoviral vector: An adenoviral vector that is depleted of all viral genes but that expresses a transgene and is packaged in a functional adenoviral capsid. The sequences necessary for replication and packaging are provided by the packaging cell line.

Herpes simplex thymidine kinase: The enzyme that phosphorylates ganciclovir into a toxic form. The gene encoding this enzyme is frequently used for suicide gene therapy.

Interleukins: Hormones that are secreted by immune cells (lymphocytes, macrophages, and dendritic cells) and affect other immune cells by attracting, activating, or inactivating them.

Liposomes: A gene delivery vehicle based on lipid vesicles that bind with DNA. Liposomes entrap DNA and allow cell membrane fusion through the lipid portion of the molecule.

p53 gene: The most commonly mutated gene identified in human cancer to date. It is a tumor-suppressor gene that protects the cells from DNA damage by sensing the damage and either stopping cell division to allow for repair or by inducing cell death (if damage is too extensive). Loss or damage of p53 allows cells to accumulate extensive chromosomal damage.

Oncogene: A gene that normally controls cell division. When the gene is deleted or altered, normal growth regulation is disrupted, predisposing the cell to malignant transformation.

Plaque-forming units: The number of viral plaques that grow on an agar plate from a given viral preparation. It is used to quantify the number of viable viruses in a given preparation to allow accurate dosing or comparisons.

Plasmid: A self-replicating extrachromosomal piece of circular DNA, normally found in bacteria, that is used for cloning and expansion.

Promoter: The DNA sequences in front of each gene that regulate the transcription of that gene. Promoters taken from viruses (such as cytomegalovirus or Rous sarcoma virus) have strong constitutive activity and are often used to drive transgenes in gene therapy vectors.

Reporter gene: Genes that are used to test the efficiency of gene transfer. Examples include genes encoding luciferase (which emits light) or β-galactosidase (which can be used to stain cells blue after addition of the proper substrate).

Suicide gene therapy: Transfer of a gene that is not normally expressed in mammalian cells and is directly toxic to the cell or activates relatively nontoxic prodrugs to a potent cytotoxic form. For example, the herpes simplex thymidine kinase gene can convert the nontoxic drug ganciclovir into a toxic analogue, thus making tumor cell susceptible to the prodrug.

Transcription: The process of converting DNA to RNA.

Transepithelial potential difference: The measurement of the voltage decrease across layers of cells (epithelia) that normally regulate tissue surface salt and water movement. This is typically performed by using a measurement electrode in the luminal space and a reference electrode in the antiluminal space. The measured voltage reflects the composite effects of electrochemical gradients across the epithelium and ion transporter activities within the epithelium. The relative contribution of different ion trans-

port pathways can be determined by carefully perfusing the luminal space with various ionic solutions and ion-transporter blockers and agonists while measuring the transepithelial potential. In cystic fibrosis, Cl^- and Na^+ transport are altered, resulting in a larger transepithelial voltage decrease in the baseline state and a reduced response to stimulators of the CFTR chloride channel.

Transfer efficiency: The percentage of cells that express the desired transgene after treatment with a gene therapy vector.

Transgene: The selected gene tested in a gene transfer experiment. For example, the herpes simplex thymidine kinase gene is the transgene used in many suicide gene therapy trials.

Translation: The process of converting messenger RNA into protein.

Vector: The vehicle by which a gene is transferred into a cell. Gene transfer vectors include viruses or liposomal conjugates.

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